Dynamics of pyrethroid resistance in a field population of *Helicoverpa armigera* (Hübner) in China

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Abstract: Dynamics of pyrethroid resistance in a field population of cotton bollworm (*Helicoverpa armigera*) was demonstrated by continuous monitoring with twin discriminating dosages, and the influencing factors were also experimentally analysed. Resistance in a field population in China increased rapidly in the 3rd and 4th generations when population density became higher and insecticides were applied repeatedly, then decreased suddenly during over-wintering and slowly in the 1st and 2nd generations when insecticide spraying was suspended. Resistance increase could be countered by dilution as a result of immigration of susceptible moths from corn fields, which were found to be a natural refuge for this pest in China. The reduction of resistance during over-wintering and the 1st and 2nd generations was affected by the lower fitness of resistant cotton bollworms to low temperature and disadvantages in reproduction. The possibilities of managing the resistance in field populations on the basis of these observations are discussed.

Keywords: pyrethroid; resistance; field population; *Helicoverpa armigera*

1 INTRODUCTION

Cotton bollworm, *Helicoverpa armigera*(Hübner), is one of the most destructive agricultural pests in the world. One particularly troublesome aspect is its ability to develop resistance to insecticides. The appearance of resistant cotton bollworm has been reported in many countries, and has caused huge economic loss and even catastrophe to cotton production. Studies on resistance management have been carried out and some reports and reviews have been published which have been helpful to our research on resistance problems in China.

Resistance of cotton bollworm to almost all kinds of chemical insecticides, including organochlorines, phosphates, carbamates and pyrethroids, has been reported in China and abroad.[3–9] In China, DDT was an effective chemical and was used extensively to control this pest. In the late 1970s resistance to DDT developed and led to failure of control in most cotton area. This, together with residue problems, finally led to abandonment of DDT in China. Organophosphates and some carbamates were then used, but most of them were not very effective against this pest, especially the aged larva. So, when pyrethroids were introduced in middle 1980s, almost all farmers used them instead. This situation led to the rapid development of pyrethroid resistance, so that in 1989, cotton bollworm was found to be resistant to pyrethroids.[9] In 1992, when there was an upsurge of this pest, pyrethroids failed to give field control, and huge economic loss to cotton production resulted. Since then studies on pyrethroid resistance management have become an active field in China.

Pyrethroid resistance in cotton bollworm seemed unstable. According to the report by Wu *et al*,[10] the extremely high resistance (>3000-fold) to fenvalerate in a laboratory-selected strain decreased to 61.4-fold after 14 generations without contact with insecticides. Some moderate resistance in field-collected strains also dropped quickly and remained at the level of 2 ~ 9-fold when reared in the laboratory, free from insecticides. Comparison of the fitness of pyrethroid-resistant and susceptible strains revealed reproductive disadvantages which made the fitness of resistant strains only 69% of that of susceptible ones.[11] This was given as the reason for resistance reduction in the laboratory.

The mechanism for pyrethroid resistance was multiple. Both higher synergism by piperonyl butoxide (PB) and increased activity in R strains confirmed that mixed function oxidase (MFO) metabolism was one of the most important mecha-

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nisms. Nerve insensitivity in R strains was also confirmed by electrophysiological studies.[12–24] Gunning *et al*[24] found reduced penetration in pyrethroid-resistant cotton bollworm. In addition, some authors have suggested that esterases contribute to the activity difference between R and S strains,[13,14,21,25] but others have found that esterase inhibitors have little synergism on pyrethroids in either R or S strain[18] So, it is still unclear whether esterases play a role in pyrethroid resistance.

Management of pyrethroid resistance in cotton bollworm has been carried out in many countries. However, China has a different farming system. The farmland is divided into very small areas and allocated evenly to each farmer. Any peasant householder can make his own decision on how to manage his areas of farmland. So, it is very difficult to adapt management tactics developed abroad. Funded by the government, we have started to explore a suitable management system. Here we report our work on demonstrating the field dynamics of pyrethroid resistance in this pest, and the factors which affect resistance development.

2 MATERIALS AND METHODS

2.1 Insects and chemical

Cotton bollworm of a susceptible strain from Xinjiang and a relatively susceptible strain from Nanjing were reared in the laboratory (28°C, L/D 16 : 8 h) for providing standard test larvae. For field resistance monitoring, suitable insects collected from host plants in various fields were mixed and used directly: younger larvae were reared to the instar required. Some time-aged larvae or moths were collected. In this case, insects were reared in the laboratory and their descendant larvae were used for tests.

Technical grade fenvalerate (98%; Sumitomo) was used as the test pyrethroid.

2.2 Resistance monitoring

Topical treatment of 4th-instar larvae was adopted for resistance monitoring. Twin dosages (1.2 μg and 0.05 μg per larva) to discriminate resistant/medium and medium/susceptible individuals, respectively, were developed, based on a multi-dosed LD-p line of a medium-resistant strain. These two doses were used simultaneously and treated 100, or 200 larvae, depending on the number of larvae collected for monitoring. The average percentage of resistant, medium and susceptible individuals were taken as the parameter reflecting the resistance state of a population.

2.3 Resistance bioassay

Topical treatment of 3rd-instar larvae was used for resistance bioassay in the laboratory. Insecticides were delivered in 0.05 μl actone to the abdominal sternum. Each insecticide was diluted into five or six gradient concentrations, and 50 larvae were treated with each concentration. Mortality was observed at 48 h. The bioassay data were analysed based on standard probit analysis. The resistance ratio was obtained by comparing the LD_{50} values.

2.4 Over-wintering experiment

Pupae of different strains were placed into plastic trays with 10 cm of moist soil, and covered by another 5 cm moist soil. The plastic trays were then enveloped with net, and put into different environmental conditions. Soil moisture was maintained by spraying water from time to time. The successfully emerged moths were observed, and over-wintering mortality was calculated.

2.5 Host-plant survey

Various crops were examined as available host plants for cotton bollworm. After consideration of both the larvae density and the area size of the host plant, the effective crop sources were deduced.

3 RESULTS

3.1 Resistance dynamics in field population

Generally, cotton bollworm breeds four generations in most cotton areas of China. The first generation feeds on over-wintered crops and causes little economic loss because the population density is very low. The moths then move to cotton fields to breed the second generation in July. The population of this generation is still small. In most case, Integrated Pest Management (IPM) can help. The 3rd and 4th generations occur overlapped in August and September, normally with higher population density, and require several sprays of insecticides.

Pyrethroid resistance in field populations was monitored with field-collected 4th-instar larvae from the 2nd to the 4th generation. By using the twin discriminating doses, the dynamics of the proportion of resistant, susceptible and the medium individuals in the population were determined (Fig 1).

In the growing season, the proportion of resistant individuals in the population decreased gradually when the use of insecticides was suspended during

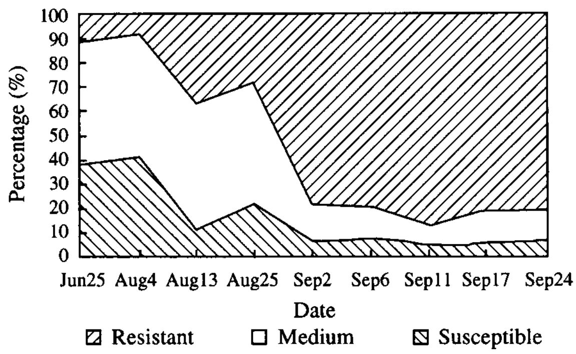

Figure 1. Pyrethroid resistance dynamics in field population of cotton bollworm.

the 1st and 2nd generations, but increased quickly again when repeated sprays of insecticides were applied late. Furthermore, comparison of the proportions at both ends of the growing season gave a hint that there was a rapid drop in resistance during the period of over-wintering. The resistance fluctuation, especially the falling-off in late August which was positively related with the proportionate areas of corn to cotton, indicate that migration dilution is occurring.

3.2 Effect of over-wintering on resistance change

Over-wintering experiments were carried out with an R (RR 43-fold) and a related S strain (RR 7-fold) of cotton bollworm. The survival rates were compared in different temperature circumstance (Table 1).

Under all the three conditions tested the S strain showed a higher survival rate than the R strain, especially under natural temperatures. The comparison of the survival rates under a constant 4°C and that in natural temperatures revealed that a fluctuating low temperature was one of the main factors causing death of the over-wintering bollworms, especially the R strain.

The over-wintering R and S strains were reared and tested for resistance (Table 2). The results demonstrated that over-wintering did not greatly affect the tolerance of the S strain to pyrethroids, but the LD_{50} of the R strain fall from 0.4 to 0.2 μg per larva and the resistance level decreased obviously, so that R cotton bollworms survived less than S ones.

3.3 Host plants and resistance dilution

When the aged surviving larva and the following moths of the same generation were collected in the same cotton field and separately reared in the laboratory, their descendants showed different levels of resistance. This suggested that there might be moths from other crop fields migrating and mixing with the population. After a survey, a large out-cotton-field population was found in a corn field, where cotton bollworm caused little economic loss and no special sprays for this pest were applied. Resistance dilution was thus deduced and demonstrated (Table 3).

Fangong was a cotton area, where insecticides were used to control cotton bollworm from the 2nd generation to the 4th. Sancang was a mixed farm area. Only the 3rd and 4th generations were controlled by chemicals. In both area, no chemical control for this pest was performed in corn fields. The percentage resistance was lower in corn fields (17% and 6% for Fangong and Sanchang resp.), and higher in cotton fields where control treatments had been used (72% and 50% resp. The next

Table 1. Comparison of the over-wintering survival rate of R- and S-cotton bollworm

Temperature circumstance	*Strain*	*No of test pupa*	*No of moths emerged*	*Percentage surviving*
Open field	S	148	72	48.6
	R	178	57	32.0
Room, natural	S	89	73	82.0
	R	40	22	55.0
Constant 4°C	S	147	128	87.1
	R	78	67	85.9

Table 2. Changes of fenvalerate resistance in R and S strains after over-wintering

Strain	*LD-p-line*	*LD_{50}(95% CL)*	*Resistance ratio*
S			
Before winter	7.1438 + 1.8451X	0.0689 (0.0464 ~ 0.1022)	7.0
After winter	6.6643 + 1.5777X	0.0881 (0.0644 ~ 0.1207)	8.9
R			
Before winter	5.6136 + 1.5765X	0.4081 (0.2963 ~ 0.5621)	41.6
After winter	6.0345 + 1.4396X	0.1912 (0.1012 ~ 0.2635)	19.5

Table 3. Proportion of resistant/medium/susceptible individuals in different populations, showing resistance dilution

Population	*Aged larvae of 3rd generation in corn field*	*Survivors of 3rd generation in cotton field*	*4th generation in cotton field before control*
Fanggong area Cotton : corn = 6 : 1	17/52/31	72/22/6	28/51/21
Sancang area Cotton : corn = 1 : 1	6/44/50	50/44/6	8/43/49

generation in the cotton field showed a much higher percentage of susceptible larvae and this seemed positively related to the relative size of the corn field (28% and 8%, resp.). In Fangong, chemical control in the 2nd generation and a lower proportion of corn fields caused a more rapid increase in the percentage resistance.

4 DISCUSSION AND CONCLUSION

Field resistance dynamics show that repeated spraying of insecticides in the late growing season is the main reason for increase in resistance. If insecticide spraying is suspended, resistance in the population will reduce in both the growing season and over-winter.

In the growing season, there are two factors leading to a reduction of resistance in the field population. The first is the lower fitness of the resistant cotton bollworm. Reproduction disadvantage in resistant strains has been demonstrated by various reporters using laboratory strains.[11,26] In our work, field resistance dynamics show that resistance in population always decreases in the early growing season when insecticide spraying is suspended. Another factor is the resistance dilution by moths from the more susceptible population outside the cotton field. These include vegetable and corn fields. In China, corn is one of the most important crops, and occupies a high proportion of farmland. Cotton bollworm feeds on the filament of corn and causes little economic loss, so no special control is performed for this pest. Corn fields thus naturally become a refuge for susceptible cotton bollworm. Our results have demonstrated its role in resistance dilution.

During over-wintering, some pupae always die, and mortality depends on environmental conditions, especially the temperature. In our experiment, higher mortality in resistant strains is followed by further reduction of resistance. This indicates that resistant cotton bollworms are less cold-tolerant than the susceptable strain.

So, if we can curtail resistance increase by reasonable use of insecticides, and accelerate the resistance reduction by utilisation of the fitness disadvantages and resistance dilution with a certain proportion of corn fields, we should be able to manage the balance of resistance increase and decrease, and prevent its further development.

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